

IMMUNOLOGIC STUDIES OF NATURAL AND EXPERIMENTAL  
CUTANEOUS STREPTOTHRICOSIS INFECTIONS IN CATTLE

by

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A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology, Parasitology  
and Public Health

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1965

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## INTRODUCTION

Dermatophilus congolensis was first identified and reported as the causative agent of cutaneous streptothricosis in cattle by Van Saceghem in 1915 (Austwick, 1958). Van Saceghem described the disease as essentially an exudative dermatitis followed by extensive scab formation. The lesions consist of raised circumscribed crust on the skin, composed of epidermal cells and coagulated serous exudate with embedded hairs.

Since 1915, outbreaks of streptothricosis have been reported in the literature from most countries of the world. The disease was first reported in the United States by Bridges and Romane in 1961 in Texas cattle. Since 1961 it has been found in New York (Bentinck-Smith et al., 1961), Iowa (Pier et al., 1963), and Kansas (Kelley et al., 1964).

Most of the literature on streptothricosis has been concerned with describing the lesions, experimental transmission, classification of the organism, theories concerning transmission and pathogenesis of the disease.

Complement fixing antibodies are produced by animals inoculated with Rhizobium (Thompson, 1954). Austwick (1958) classified Rhizobium as a synonym for Dermatophilus pedis and placed it in the same genus as D. congolensis. However, Gordon and Edwards (1963) placed D. pedis and D. dermatonomus (etiological agent of mycotic dermatitis in sheep) as strains of D. congolensis.

There is conflicting evidence on the development of immunity to streptothricosis (Ainsworth and Austwick, 1959). Hudson (1937)

reported the resistance of rabbits to reinoculation. However, Nisbet and Bannatyne (1955) found no evidence of immunity. Mémerý and Mémerý (1962) stated that agglutins are produced by infected animals.

In this study, agar gel precipitation, indirect hemagglutination and agglutination tests were adapted to study the immunologic responses in cattle from natural and experimental infections.

#### REVIEW OF LITERATURE

Early reports. Following Van Saceghem's early reports of Dermatophilus congolensis, there were few additional reports of cases specifically diagnosed as Dermatophilus infections until the late 1930's. However, Armfield (1918), Griffiths (1918), and Hornby (1920), in Africa, reported mycotic conditions in cattle which were believed to have been caused by Dermatophilus. The disease was then known by a variety of names. Lane in 1915 reported it as "Senkobo scab"; Curson in 1920 called it "Saria"; and in other areas of Africa it was also known as "Drodro-Boka," "Ushin-Gishu skin disease," and "Impetigo contagieux" (Austwick, 1958). Henderson in 1927 applied the term streptothricosis which has been adopted in almost all English and French publications (Austwick, 1958). In addition to being found in Africa, streptothricosis has been reported in Australia, New Zealand, India, and Europe (Ainsworth and Austwick, 1959). Other than in parts of the United States, the most recent additional initial isolation



reports of streptothricosis are those of Perez Catan and di Rocco (1963 a, b) from South America.

Gross lesions. Van Saceghem identified the disease clinically by five signs: (1) formation of crusts over which the hairs were erect; (2) rapid spread of the disease over the animal; (3) complications which might bring about death; (4) young animals and adults affected to the same degree; and (5) a seasonal affection which was observed in the acute form during the rainy season (Griffiths, 1918).

Schulz (1955) gave one of the few reports which described the early signs as being marked lacrymation and a secretion from the nostrils which preceded the skin eruption. The early skin lesions were described as patches of slight erythema by Chodnik (1956). In the next stage a small amount of exudate appears on the skin giving the affected areas a greasy, matted appearance. At this stage the condition resembles an eruptive and exudative eczema (Schulz, 1955). The exudate coagulates to form a yellowish brown crust and hard scabs form which adhere to the skin. The hair becomes matted together by the dried exudate and stands erect to form the "paint brush" lesions (Hornby, 1920). Subsequently, these elevations increase in size and become confluent to form thick irregular scabs and wart-like structures. These are firmly adherent to the tissue at first but later they may be fairly easily detached by scraping or traction. In the latter case a pinkish, moist surface with a greyish exudate is exposed. Profuse bleeding occurs and a raw base remains on removal of the

adhesive scab by force. The crust is composed of hair, epidermal cells and coagulated exudate, and has a concave base (Ainsworth and Austwick, 1959).

Histopathology. The histopathologic changes seen as the result of D. congolensis infections are mainly limited to the skin (Chodnik, 1956). In the early stages the epithelium becomes acanthotic due to proliferation of the prickle cells, and there is evidence of intracellular and intercellular edema (Schulz, 1955). This is followed by an infiltration of mononuclear and polymorphonuclear cells from the dermis which gives rise to pustules and microabscesses (Bridges and Romane, 1961). Exudate mats the hair together forming the so-called "paint brush" lesions which consist of the dried exudate mixed with masses of desquamated epithelial cells and tangled mycelial elements of the organism (Chodnik, 1956). Many narrow branching hyphae are seen in the heavy keratin layers of the skin and in the hair follicles. These hyphae do not grow into the dermis, but frequently appear in microabscesses in the dermis where the hair follicles have undergone necrosis (Bridges and Romane, 1961).

Cultural characteristics. Roberts (1961) in studying the life cycle of D. dermatonomus by use of the light microscope, and Gordon and Edwards (1963) in studying the life cycle of D. congolensis by use of electron microscopic study of ultrathin sections, observed essentially the same development of the organism from the motile cocci. They found that the filaments arise as tubular protrusions resembling germ tubes from the cocci.

As the tube grows, transverse septation occurs 5 to 30 microns behind the tip of the growing hyphae. Secondary and successive transverse and longitudinal septa are formed 0.3 to 0.5 micron apart which give rise to cocci and packets of cocci. The coccoid elements are embedded in a gelatinous matrix from which they may escape, assume motility, and thus complete the cycle. Alternatively, the cocci remain attached and germinate in situ.

The cocci are variable in size (0.5 to 1.5 microns) and stain Gram-positive. The branched filaments, composed of parallel rows of cocci in a gelatinous matrix, may be up to 5.0 microns wide. These filaments form the tangled mycelium seen in cultures and in natural lesions.

Epidemiology. The organism is resistant to drying (Roberts, 1957a) and will remain viable in scab material for as long as 2½ years (Roberts and Vallely, 1962). Therefore, infective material would have widespread distribution in the vicinity of any infected animal.

Though the epidemiology of streptothricosis is not fully understood, it appears that an animal's general or local resistance must be reduced in order to establish an infection and subsequent disease (Ainsworth and Austwick, 1959; Macadam, 1964). Factors which have been implicated in streptothricosis outbreaks include: (1) prolonged wetting of the skin (Chodnik, 1956; Roberts and Vallely, 1962); (2) skin wounds (Austwick and Davies, 1958); (3) tick bites (Plowright, 1956; Macadam, 1962); (4) thorns (Zlotnik, 1955); (5) Demodex infestation (Mornet and Thiéry,



1955); and (6) breed susceptibility (Hornby, 1920; Kelley et al., 1964).

Experimental studies. In the experimental studies horses, cattle, goats, sheep, rabbits and guinea pigs have been experimentally infected (Mason and Bekker, 1934; Edgar and Keast, 1940; Dean et al., 1961; Egerton, 1964). These experimental lesions persist from a few days to a few weeks before spontaneously healing. Macadam (1961) found that high humidity did not predispose experimental animals to generalized streptothricosis. In Africa, Plowright (1956) found that dipping cattle in gammexane, as a control measure for ticks, was also effective in preventing streptothricosis. Stableforth (1937), Snijders and Jansen (1955), Simmons (1955), Roberts (1957b), Plowright (1958) and Pier et al. (1963) compared the biochemic and morphologic characteristics of the Dermatophilus species. They found only slight biochemical and growth rate variations and very little morphologic difference among the isolates studied.

The reports of Thiéry and Mémery (1961) and Mémery and Mémery (1962) are the only reports in the literature of the presence of circulating antibodies in animals infected with D. congolensis. However, in their reports they do not give the test reactions used to detect the agglutins or the magnitude of the reactions found in infected animals.

In studies of an organism from sheep called Rhizobium, Thompson (1954) used complement fixation reactions to show that antibodies had been produced by inoculated rabbits. Austwick



(1958) classified Thompson's Rhizobium as a synonym for Dermatophilus pedis and Gordon and Edwards (1963) classified it as a strain of D. congolensis. Other reports of immunity have been based on resistance to reinoculation (Hudson, 1937; Austwick and Davies, 1958). However, the opposite conclusion, no immunity, had been drawn when animals were susceptible to reinfection (Stableforth, 1937; Nisbet and Bannatyne, 1955).

Diagnostic methods. Recognition of cutaneous streptothricosis has depended largely upon the appearance of lesions in clinically diseased animals, microscopic identification of typical filaments, and culture of the causative organism, D. congolensis, in exudative crusts. Cultural isolation of D. congolensis is often unrewarding due to the rapid overgrowth of contaminating saprophytes and secondary invaders. Hudson (1937) desiccated the crusts for 9 days over concentrated sulfuric acid to reduce the bacterial contamination. Macadam (1961) flamed the crusts prior to selecting material for culture from the concave base of the crust. Hudson (1937) and Mémerly (1961) applied ground exudative crusts to shaved, scarified areas and to areas from which the hair had been plucked. The organisms were isolated in nearly pure culture from the lesions that developed in 4 to 7 days on the rabbits. Roberts (1963a) and Pier et al. (1964) cultured filtrates from suspensions of the ground crusts. The 1.2  $\mu$  pore size filter allowed passage of D. congolensis but retained most of the contaminants. Kelley et al. (1964) used 1,000 units of Polymixin B per ml. of medium to control contaminants.

Kelley et al. (1964) used 1,000 units of Polymixin B per ml. of medium to control contaminants. Pier et al. (1964) used fluorescent antibody techniques to detect the organism in exudate suspensions.

Immunology. Although the organism has many synonyms and has been classified as both fungi and bacteria, current literature seems to be in agreement that the organism is a member of the order Actinomycetales (Hesseltine, 1960; Austwick, 1958). Serological methods can play an important part in diagnosis, prognosis and management of infections caused by pathogenic organisms of the order Actinomycetales (Salvin, 1959). Agglutinins, precipitins, hemagglutinins, and complement fixing antibodies are produced by animals infected with the pathogenic Actinomycetales (Salvin, 1959). However, the serologic methods have not been sufficiently studied and standardized to be in routine use.

Agglutination and precipitin reactions have been developed using ground suspensions and sonically disrupted suspensions of the organism for antigens. Complement fixation and hemagglutination tests also have been useful aids in diagnosis and prognosis of Actinomycetales infection (Savin, 1959).

In this study gel diffusion, indirect hemagglutination, and agglutination reactions were selected for their reproducibility, sensitivity, and general accepted useability as methods of demonstrating antibodies.

Passive hemagglutination reactions with tannic acid treated red blood cells have been shown to be specific, highly sensitive

means of detecting antibodies. With some antisera, 0.001 micrograms of antibody can be detected (Stavitsky, 1954b).

Immunodiffusion, although first described in 1905, did not become widely employed until 1946. Since then, however, immunodiffusion has been widely used in detecting and analyzing immunologic reactions (Crowle, 1961).

Agglutination of organisms in the presence of an immune serum has been observed and studied since the 19th century. The agglutination reactions have been widely used in medicine and related sciences.

#### MATERIALS AND METHODS

##### Culturing and Harvesting of Dermatophilus congolensis

Throughout the experiments, 4 isolates of D. congolensis, from 3 infected Shorthorn cattle and 1 from an infected Holstein cow, were used as the source of organisms. Each isolate was grown on defibrinated 10% sheep blood agar plates containing 1000 units of Polymyxin B sulfate<sup>1</sup> per ml. of medium. Broth, composed of 1% Tryptone,<sup>2</sup> and 0.5% sodium chloride was inoculated with colonies picked from the blood agar plates. The broth cultures were incubated at 37° C for 7 to 14 days before harvesting. The cocci and filaments were harvested from the broth cultures by centrifugation for 20 minutes at 1000 g. A portion of each

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<sup>1</sup> Chas. Pfizer, Inc., New York, N. Y.

<sup>2</sup> Difco Laboratories, Detroit, Mich.



preparation was streaked on blood agar to check for contamination. The organisms were then washed and recentrifuged 3 times with phosphate buffered saline (PBS) solution 0.15M, pH 7.4. This was the basic method used to grow the organism used for subsequent procedures.

### Hyperimmune Serum

The experimental animals were hyperimmunized to provide the positive serum used in the development of the immunologic reactions for streptothricosis.

Experimental Animals. In order to produce hyperimmune serum 8 rabbits, 3 Holstein calves, and 2 Shorthorn calves were used. The 2 Shorthorn calves were animals which had recovered from a natural infection of streptothricosis. The other animals had had no previous contact with the disease. From this group of animals 1 Holstein and 1 Shorthorn calf and 2 rabbits were used as the control animals. Two antigen and 2 control preparations were made.

Antigen I. A 50 ml. suspension of washed cocci and filaments (prepared as described in Culturing and Harvesting of D. congolensis) was suspended in 0.015M phosphate buffered saline, pH 7.4, and was standardized to an optical density of 0.37 at 460 m $\mu$  in a spectrophotometer.<sup>3</sup> This suspension was subjected to

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<sup>3</sup> Coleman Junior Spectrophotometer, Model 6C, Coleman Instruments, Inc., Maywood, Ill.



sonic waves for 70 minutes in a 20 k.c. oscillator.<sup>4</sup> Examination of stained films, made after sonic oscillation, revealed mostly cellular debris; however, some cells still appeared to be intact. This partially disrupted cellular solution was preserved with 0.01% thimerosal,<sup>5</sup> and was used as Antigen I.

Antigen II. This antigen was prepared from a washed suspension of the organisms (prepared as described in Culturing and Harvesting of D. congolensis) standardized to an optical density of 0.37 at 460 mμ. Antigen II was inactivated and preserved with 0.5% phenol.

Control Preparations. Control preparation I consisted of a 1/50 dilution of the medium in PBS, pH 7.4, with 0.01% thimerosal. Control preparation II consisted of a 1/50 dilution of the medium in PBS, pH 7.4, with 0.5% phenol. The experimental animals were given 1 intradermal and 1 subcutaneous injection each week for 4 weeks. The injections given are shown in Table 1.

Serum was collected prior to injection, and then 15 and 30 days after the last injection. The cattle were bled by venipuncture, and the rabbits were bled by cardiac puncture while under ether anesthesia.

#### Whole Cell Tube Agglutination Test

Particulate Antigen for Agglutination. Approximately 20 grams (wet weight) of the washed cells and filaments (prepared as

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<sup>4</sup> Model LS - 75 Sonifier, Branson Sonic Power, Div. of Branson Instruments, Inc., Danbury, Conn.

<sup>5</sup> Merthiolate, Eli Lilly & Co., Indianapolis, Ind.

TABLE 1—Hyperimmunization Procedure

Number of animals	First and third week		
	Amount injected	Material injected	Route of injection
6 rabbits	0.1 ml.	Antigen I	Intradermal
Group I	0.5 ml.	Antigen I	Subcutaneous
2 rabbits	0.1 ml.	Control I	Intradermal
Group II	0.5 ml.	Control I	Subcutaneous
3 calves	0.2 ml.	Antigen I	Intradermal
Group III	2.0 ml.	Antigen I	Subcutaneous
2 calves	0.2 ml.	Control I	Intradermal
Group IV	2.0 ml.	Control I	Subcutaneous
Second and fourth week			
6 rabbits	0.1 ml.	Antigen II	Intradermal
Group I	0.5 ml.	Antigen II	Subcutaneous
2 rabbits	0.1 ml.	Control II	Intradermal
Group II	0.5 ml.	Control II	Subcutaneous
3 calves	0.2 ml.	Antigen II	Intradermal
Group III	2.0 ml.	Antigen II	Subcutaneous
2 calves	0.2 ml.	Control II	Intradermal
Group IV	2.0 ml.	Control II	Subcutaneous

described in Culturing and Harvesting of D. congolensis) were suspended in 200 ml. of 0.15M sterile saline solution by mixing for 5 minutes in a blender.<sup>6</sup> The material was then standardized at an optical density of 0.95 at 460 mμ. The organisms were inactivated by adding phenol to a final concentration of 0.5%.

<sup>6</sup> Model 700B Waring Blender, Waring Products Corp., Winsted, Conn.

Test Procedure. Agglutination tests were conducted by adding 0.5 ml. of the particulate antigen preparations to 13 x 100 mm. tubes containing 0.5 ml. serial twofold dilutions of the test serum in 0.15M saline solution. These preparations were thoroughly shaken and placed in a 37° C water bath for 48 hours. Antigen controls in saline and known positive serum were included each time the reaction was run. Readings from negative to 3 + reactions were graded as follows:

- + + + smooth mat on bottom of tube with folded edges
- + + smooth mat covering entire bottom of tube
- + smooth mat covering most of the bottom of tube
- small discrete ring or button in center of bottom of tube

#### Agar Gel Precipitation Reactions

The agar gel diffusion technique of Ouchterlony (Crowle, 1961) was followed in principle.

Diffusion Plates. A 1.5% solution of Noble's Special Agar<sup>7</sup> was prepared containing 0.01% thimerosal. Sixty ml. of agar were poured into 100 x 15 mm. plastic petri dishes. After the plates had hardened, 0.5 cm. diameter holes were cut with a sterile cork bore. The agar plugs were removed, and the bottoms of the wells were sealed with additional melted agar. The distance between the central well and peripheral wells was 0.5 cm.

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<sup>7</sup> Difco Laboratories, Detroit, Mich.

Soluble Antigen I for Gel Diffusion. Approximately 8 grams (wet weight) of the final precipitate of washed cells and filaments (prepared as described in Culturing and Harvesting of D. congolensis) were mixed with 3 grams of fine glass particles and placed in a sterile mortar and pestle. The mixture was cooled to  $-15^{\circ}$  C. After freezing, the mixture was ground as it thawed. The freezing, thawing, grinding procedure was repeated 12 times. After grinding, no intact cells could be found on examination of stained smears. The ground cell suspension was washed from the mortar and suspended in 10 ml. of PBS.

Soluble Antigen II for Gel Diffusion. This antigen was prepared from washed cells and filaments which had been subjected to sonic waves for 70 minutes in a 20 k.c. sonic oscillator (prepared as described in Antigen II under Hyperimmune Serum).

Test Procedure. The peripheral wells were filled with 0.15 to 0.2 ml. of serum, and the central well was filled with 0.15 to 0.2 ml. of antigen. Alternatively, the peripheral walls were filled with antigen and the central well was filled with serum. The plates were kept at  $37^{\circ}$  C and the precipitation lines were recorded daily for 7 to 10 days.

#### Indirect (Passive) Hemagglutination Test

Essentially, Stavitsky's (Stavitsky, 1954a) modification of Boyden's (Boyden, 1951) hemagglutination test of tannic acid treated red blood cells was followed.

Soluble Antigen. A soluble antigen was prepared by freezing, thawing, and grinding of D. congolensis (as described in Soluble



Antigen I for Gel Diffusion) except that ground cell suspension was washed from the mortar and suspended to a final volume of 30 ml. with PBS. This suspension was centrifuged for 20 minutes at 1000 g to remove insoluble cellular debris and glass particles. The supernatant was used as the soluble antigen for hemagglutination reactions.

Antigen Coated Red Blood Cells. Red blood cells from 3 ml. blood were washed 3 times with 10 ml. of saline. After the final centrifugation for 5 minutes at 500 g, 0.5 ml. of the packed, washed, red blood cells were suspended in 20 ml. of buffered saline, pH 7.2. Twelve ml. of the red blood cell suspension were mixed with 12 ml. of a 1/20,000 dilution of tannic acid and incubated at 37° C for 10 minutes. The tannic acid treated red blood cells were centrifuged for 5 minutes at 500 g. The supernatant was decanted, and the red blood cells were resuspended twice in 12 ml. of buffered saline, pH 7.2. To coat the tanned red blood cells 40 ml. of buffered saline, pH 6.4, plus 10 ml. of the soluble antigen and 10 ml. of the tanned red blood cells were mixed in this order and held at room temperature for 10 minutes. The cells were then centrifuged, washed once with 20 ml. 1/100 dilution of inactivated, absorbed, normal rabbit serum, and were suspended in 10 ml. of 1/100 dilution of this rabbit serum. This final 10 ml. suspension of antigen coated red blood cells was used as the antigen. If not used immediately, this antigen preparation was kept at 5° C for not more than 18 hours.

Test Procedure. Prior to use, normal rabbit serum and all test serums were inactivated in a 56° C water bath for 30 minutes

and then absorbed with an equal volume of washed sheep red blood cells for 10 minutes at room temperature.

Serial twofold dilutions of test serum were prepared using a 1/100 dilution of normal rabbit serum in saline. Each 13 x 100 mm. dilution tube contained 0.5 ml. of diluted serum. Diluted normal rabbit serum is necessary to prevent autoagglutination of the tannic acid treated red blood cells (Campbell et al., 1963).

After 0.05 ml. antigen coated red blood cells were added to the dilutions of test serums, the tubes were shaken and kept at room temperature. Reactions were read after 12 hours. The titer was expressed as the reciprocal of the highest dilution of serum which gave a definitely positive reaction. The reactions were graded from negative to 3 + as follows:

- + + + smooth mat on bottom of tube with folded or ragged edges
- + + smooth mat covering entire bottom of tube
- + narrow ring of red around edge of smooth mat, covering most of bottom of the tube
- + smaller area of tube covered than +, with heavier ring around edge
- discrete red ring or button in center of the bottom of tube

Controls. Control tannic acid-saline cells were prepared by substituting saline for the soluble antigen in the above procedures. This control, as well as known positive serum and serum diluent controls, was included each time the reaction was run.

## Experimental Streptothricosis Infections

Experimental Animals. Seven Holstein calves, 8 months of age, were used in this portion of the investigation. One calf was kept in an isolated stall as a control. Another control calf was kept in the same pen with the 5 infected animals.

Preparation of Areas for Inoculation. Four areas 10 cm. x 15 cm. on the back of each calf were clipped. Sixteen of these areas were swabbed with irritating chemicals in an attempt to make the skin more susceptible to the infection. The following chemicals were used: 1%, 5% and 10% sodium hydroxide, 1% hydrochloric acid, 1% sulfuric acid and xylene. The areas were swabbed 3 times at 48-hour intervals.

Inoculation. Five days after the last application of the irritants the areas were rinsed with water, locally anesthetized with 2% procaine, scarified and inoculated with mixed 1% Tryptone broth suspensions of the 4 isolates of D. congolensis. Each area was swabbed with 10 ml. of this broth suspension which contained 27 million organisms per ml., as determined by using standard plate counting methods with a medium composed of 1% Tryptone, 0.5% sodium chloride and 2% agar. On the 4 areas which had not received applications of the irritants, 2 were swabbed with 1% histamine<sup>8</sup> and 2 with 150 units of hyaluronidase<sup>9</sup> after scarification and prior to inoculation.

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<sup>8</sup> Histamine diphosphate, Nutritional Biochemical Corporation, Cleveland, Ohio.

<sup>9</sup> Wydase, Wyeth Laboratories Inc., Philadelphia, Pa.



Collection of Specimens. Serum samples were collected before inoculation and 3 times per week for 5 weeks after inoculation. Specimen scab material was collected from the areas twice a week for 5 weeks after inoculation. Two weeks after inoculation a skin biopsy from a lesion area, measuring approximately 0.5 cm. x 2 cm. in size, was removed from each calf and placed in 10% formalin for histopathologic examination. Sections were stained with hematoxylin and eosin and with Grocott's fungus stain.

Microbiology. A portion of the specimen scab material collected from the lesions on each calf was ground using a sterile mortar and pestle. The ground material was inoculated onto 10% defibrinated sheep blood agar plates containing 1000 units of Polymyxin B sulfate per ml. of medium and incubated at 37° C for 7 days. Another portion of the scab material was examined by soaking in sterile saline and smearing on glass slides. The slides were fixed in methyl alcohol for 5 minutes and stained with Giemsa stain for 20 minutes. The smears were examined with the oil immersion objective (1200X).

#### Naturally Infected Cattle

In an infected Shorthorn herd, serum samples were collected from 1 calf with severe lesions, 4 calves with slight lesions, and from 4 calves that did not show lesions. Serum samples were also collected from an infected Shorthorn calf from a second herd. Serum samples were taken monthly for 10 months from 2 cows which recovered from natural streptothricosis infections. These serums were stored at -15° C until tested.



## RESULTS

### Cultural Characteristics

On blood agar, all isolates of D. congolensis were slow growing and produced 1 to 5 mm. zones of beta hemolysis around the colonies. The colonies were visible after 24 hours incubation at 37° C. However, 4 to 7 days incubation was necessary before the typical yellowish, verrucose, dry colonies, which indented in the agar, were fully developed. In the 1% Tryptone and 0.5% sodium chloride broth, 3 isolates formed typical cottony balls, most of which were tightly adherent to the walls of the tube. The fourth isolate formed a pellicle in the broth and clouded the medium. The fifth isolate, which was used to check the specificity of the immunologic reactions, also formed the adherent cottony balls.

### Agglutination Reactions

Particulate antigen suspensions prepared from 4 isolates of D. congolensis were used to test for agglutinins in the serums of experimentally infected and naturally infected cattle. When first using this antigen with the serums from rabbits and cattle repeatedly inoculated with killed suspensions of D. congolensis, it was found that an agglutination reaction could be elicited (Table 2). No reaction was seen with the serum from normal rabbits. Serums from infected cattle had agglutination titers of 1:160 to 1:640 (Table 3). However, serums from normal cattle had agglutination titers of 1:10 to 1:20.

TABLE 2—Immunologic Reactions of Hyperimmunized Animals

Group	Animal number	Preinjection			Post injection		
		Hemaggl.*	Aggl.*	Gel-dif.**	Hemaggl.	Aggl.	Gel-dif.
Group I (6 hyper-immunized rabbits)	3	-	-	-	160	160	+
	4	-	-	-	80	160	+
	5	-	-	-	320	320	+
	6	-	-	-	320	320	+
	7	-	-	-	320	320	+
	8	-	-	-	160	320	+
Group II	1	-	-	-	-	-	-
(2 control rabbits)	2	-	-	-	-	-	-
Group III	426	20	10	-	640	640	+
(3 hyper-immunized calves)	427	20	10	-	640	640	+
	207	320	160	+	2560	640	+
Group IV	432	20	10	-	20	10	-
(2 control calves)	20	640	320	+	640	320	+

\* Passive hemagglutination and whole cell agglutination titers expressed as reciprocal of original serum dilution.

\*\* Agar gel precipitation, - = no precipitation lines, + = precipitation lines.

Calves 207 and 20 were animals which had recovered from natural infections of streptothricosis.

TABLE 3—Immunologic Reactions in Normal Cattle and in Cattle Naturally Infected with Dermatophilus congolensis

Animal number	Infection status	Hemaggl.*	Aggl.*	Gel-dif.**
D70	Severe natural infection	2560	640	+
D77	Natural infection	640	160	+
D73	Natural infection	1280	320	+
D69	Natural infection	80	320	+
D72	Normal	40	20	-
D78	Normal	20	10	-
D74	Normal	40	20	-
D81	Normal	20	10	-
2329	Normal	20	10	-
210	Chronic natural infection of 8 months duration	640	320	+
210	Chronic natural infection of 18 months duration	160	80	+
207	4 months after natural infection	320	160	+
207	12 months after natural infection	320	80	+
20	Natural infection	640	320	+
20	8 months after natural infection	320	160	+

\* Passive hemagglutination and whole cell agglutination titers expressed as reciprocal of original serum dilution.

\*\* Agar gel precipitation, - = no precipitation lines, + = precipitation lines.

The specificity of the agglutination reaction was tested by adsorbing the serum with a washed suspension of the fifth isolate of D. congolensis overnight at 37 C. The cells were then removed by centrifugation, and the serum was tested with a particulate antigen. Untreated serum agglutinated the antigen suspension; but after adsorption, the serum gave no reaction.

#### Agar Gel Precipitation Reactions

Lines of precipitation appeared in 24 to 72 hours when the serums of the hyperimmunized rabbits and cattle were tested against Soluble Antigen I (frozen and ground) and against Soluble Antigen II (sonically disrupted). Similar lines of precipitation were seen from the serums naturally infected and experimentally infected animals. The lines did not appear until 5 to 7 days in serums from the less severely infected animals and in the serums from recovered animals. When both antigens were tested against the same hyperimmune serum, the precipitation lines formed a continuous band around the serum well indicating common antigenic material in both antigen preparations. With Soluble Antigen I the precipitation lines appeared more quickly and were more intense than those seen with Soluble Antigen II. When hyperimmune serums were adsorbed with washed suspensions of the fifth isolate of D. congolensis and then tested with the antigens I and II, only very faint precipitation lines could be discerned. When control preparations I and II (hyperimmune serum experiment) were tested against homologous and heterologous serums, no precipitation lines were seen.



Common reactions, as evidenced by formation of a continuous band, were observed when serums from naturally infected animals, experimentally infected animals, and hyperimmunized animals were placed in the peripheral wells and the soluble antigen was placed in the center well.

#### Passive Hemagglutination

Soluble material from D. congolensis suspensions, adsorbed onto tanned red blood cells, was used to test for antibodies in the serums of hyperimmune animals, experimentally infected calves and naturally infected cattle. In dilutions from 1:80 to 1:2560, the hyperimmune serums from rabbits and calves agglutinated the antigen adsorbed tanned red blood cells. Serums from experimentally infected cattle had hemagglutination titers from 1:320 to 1:2560 (Table 4). Serums from naturally infected cattle had hemagglutination titers from 1:160 to 1:2560 (Table 3). Serums from normal cattle had hemagglutination titers from 1:20 to 1:40. Normal rabbit serums did not agglutinate the test antigen.

The hemagglutination reactions were inhibited when the serums were adsorbed with washed suspensions of the fifth isolate of D. congolensis prior to testing by passive hemagglutination reactions. Some variations in reproducibility of the titer in the same serum sample occurred with successive lots of antigen for hemagglutination.

TABLE 4—The Appearance of Immunologic Reactions in Cattle During the Course of Experimental Infection with Dermatophilus congolensis.

Animal	Pre-inoculation	Weeks post inoculation				
		1	2	3	4	5
434						
Hemagglutination*	20	20	320	640	1280	2560
Agglutination*	10	10	80	160	320	320
Gel-diffusion**	-	-	+	+	+	+
433						
Hemagglutination	20	20	640	1280	2560	2560
Agglutination	10	10	160	320	640	640
Gel-diffusion	-	-	+	+	+	+
430						
Hemagglutination	40	20	160	320	320	320
Agglutination	10	10	40	160	160	160
Gel-diffusion	-	-	-	+	+	+
429						
Hemagglutination	20	10	160	160	320	640
Agglutination	10	20	80	80	320	640
Gel-diffusion	-	-	+	+	+	+
201						
Hemagglutination	20	20	320	640	1280	1280
Agglutination	10	10	80	160	320	320
Gel-diffusion	-	-	+	+	+	+
428						
Hemagglutination	20	20	20	20	20	20
Agglutination	10	10	10	10	10	40
Gel-diffusion	-	-	-	-	-	-

\* Passive hemagglutination and whole cell agglutination titers expressed as reciprocal of original serum dilution.

\*\* Agar gel precipitation, - = no precipitation lines, + = precipitation lines.

### Naturally Infected Cattle

The immunologic reactions from naturally infected cattle are shown in Table 3. Positive immunologic reactions persisted for 18 months in a chronically infected cow and for 8 and 12 months in cattle which had recovered from natural streptothricosis infections. In each case these were the longest periods tested.

### Experimental Streptothricosis Infections

Cutaneous streptothricosis lesions developed on all 5 of the inoculated calves. D. congolensis were demonstrated in Gemisia stained smears from the scabs and on blood agar cultures from scab material (Table 5) throughout the 5-week experimental period.

The areas which had received applications of 5% and 10% sodium hydroxide developed lesions only around the peripheries of the irritated areas. The lesions on the other areas covered the irritated sites, and some of these lesions spread 2 to 3 cm. around the area during the course of the infections.

Within 7 days after inoculation the areas were covered with soft scales and soft scabs. When these scabs were removed, the underlying area was moist and had pin-point hemorrhages. Two weeks after inoculation the crust and scabs were built up to 0.5 to 1.0 cm. thickness. Removal of the scab left a moist bleeding area. During removal, the scabs came off as a sheet or as a circular scab with a concave base. During and after the third

TABLE 5—Results of Blood Agar Cultures and Gemisia Stained Smears from Experimentally Produced Lesions of Streptothricosis

Animal	Chem. applied preinoculation	Weeks post inoculation									
		1		2		3		4		5	
		C*	S**	C	S	S	C	S	C	S	C
434	Areas										
	A 10% NaOH	-	-	-	-	+	+	+	+	-	-
	B 1% H <sub>2</sub> SO <sub>4</sub>	+	+	+	+	-	-	-	+	-	-
	C Xylene	+	+	+	+	+	+	+	+	+	+
	D 5% NaOH	-	-	+	+	-	+	-	+	-	-
433	Areas										
	A 10% NaOH	-	-	+	+	+	+	-	-	-	-
	B 1% H <sub>2</sub> SO <sub>4</sub>	+	+	+	+	+	+	-	+	-	-
	C Hyaluronidase	+	+	+	+	+	+	+	+	+	+
	D 1% histamine	+	+	+	+	+	+	+	+	+	+
430	Areas										
	A 10% NaOH	-	-	-	+	+	+	-	+	-	-
	B 1% H <sub>2</sub> SO <sub>4</sub>	+	+	+	+	+	+	+	+	+	+
	C Hyaluronidase	+	+	+	+	+	+	+	+	+	+
	D 1% histamine	+	+	-	+	-	+	+	+	+	+
429	Areas										
	A 1% HCl	+	+	+	+	+	+	+	+	+	+
	B 1% NaOH	+	+	+	+	+	+	+	+	+	+
	C Xylene	+	+	+	+	+	+	+	+	+	+
	D 5% NaOH	-	-	+	+	-	+	-	-	-	-
201	Areas										
	A 1% HCl	+	+	+	+	-	+	+	+	+	+
	B 1% NaOH	+	+	+	+	+	+	+	+	+	+
	C Xylene	+	+	+	+	+	+	+	+	+	+
	D 5% NaOH	-	-	+	+	-	+	-	-	-	-

\* Cultured on 10% blood agar, polymyxin B plates.

\*\* Gemisia stained scab smears.

+ - Isolation and identification of D. congolensis by culture or Gemisia stained smears.



week, post inoculation, the original scabs were gradually shed. Secondary scabs and crust formed in the areas where the original scabs had been shed.

These secondary scales continued to build up and form heavy scabs. Most of the original scab lesions had been shed by the fifth week, post inoculation. However, 12 of the 20 areas were still partially covered with secondary scab lesions which were firmly attached and were spread over the areas.

### Histopathology

Histopathologic examination of skin biopsies revealed the following: D. congolensis in the epithelial crusts, in microabscesses in the stratum corneum, in the hair follicles, and in microabscesses around the hair follicles in the dermis. The presence of D. congolensis in the hair follicles was accompanied by mild to pronounced inflammatory changes. These areas were infiltrated with neutrophiles, lymphocytes, macrophages, fibroblasts, and occasional eosinophiles and plasma cells. In the infected areas the epithelium was acanthotic, and hyperkeratosis and parakeratosis were evident.

The crusts and scabs covering the epithelium were composed of debris consisting of the following: keratohyalin material, parakeratotic cells, necrotic leukocytes, masses of hyphae and other debris.

## DISCUSSION

Circulating antibodies in experimentally and naturally infected cattle were demonstrated by whole cell agglutination, passive hemagglutination, and agar gel-diffusion tests in this study. These immunologic responses persisted for 18 months in a chronically infected cow and for 8 and 12 months in cattle which recovered from natural infections (longest periods tested).

Although the causative agent of streptothricosis, D. congolensis, is antigenic and infected animals respond to this organism by antibody production, these antibodies are not completely protective. The chronic nature of the disease and the fact that some animals can be reinfected indicates that antibody production alone cannot effect recovery or prevent reinfection.

Although the organism has not been found as a saprophyte in the soil or on the hair coat of normal animals, saprophytic existence has been postulated and investigated by Mason and Bekker (1934), Ainsworth and Austwick (1959), and Roberts (1963b). The presence of low levels of antibodies in the serum of normal cattle which have had no known contact with the organism lends support to the possibility of a saprophytic existence of the organism.

However, these low titer hemagglutination and agglutination reactions may also be due to cross reactions or nonspecific agglutination. Many agglutinins in the serum from apparently normal cattle are observed in other immunologic reactions.

The antigens involved in the passive hemagglutination and agglutination reactions are believed to be somatic surface or flagellar antigens because of the ability of whole cells to remove agglutinins from serum by absorption. As in the case of higher bacteria and fungi, D. congolensis may have many antigenic fractions. It is a highly complex differentiated organism.

The demonstration of antibodies by immunologic reactions in rabbit serum after repeated injections with suspensions of D. congolensis is similar to results produced by Pier et al. (1964). He collected gamma globulin fractions from hyperimmunized rabbits for use in fluorescent antibody reactions. Memery and Memery (1962) found that even though antibodies were produced by hyperimmunized rabbits, these antibodies did not protect the rabbits against reinfection. The lack of complete immunity, even with circulating antibodies present, has been shown in other infections with dermatophytes (Lewis et al., 1958).

Experimental infections typical of streptothricosis were produced in 5 calves on areas which had been irritated and scarified prior to inoculation. These experimentally produced lesions persisted longer than experimental infections previously reported.

The histopathologic changes were similar to those seen in natural infections as described by Schulz (1955), and Bridges and Romane (1961).

Since the organism is antigenic, serological methods could be used to study the antigenic relationships of the D. congolensis strains and resolve the difference in opinions regarding the

proper species classification of the *Dermatophilus* isolates.

These passive hemagglutination, agglutination, and agar gel precipitation reactions can be used to augment the present cultural and microscopic methods of diagnosing cutaneous streptothricosis.

### CONCLUSIONS

Experimentally and naturally infected cattle produce circulating antibodies which can be demonstrated by passive hemagglutination, whole cell agglutination, and agar gel precipitation techniques. Cultural and microscopic methods of diagnosing *D. congolensis* infections can be augmented by using these immunological tests.

In experimental infections the use of irritants before inoculation contributed to more persisting lesions of experimental streptothricosis than previously reported in the United States.



### ACKNOWLEDGMENTS

The author wishes to express his gratitude to Doctor D. C. Kelley, Associate Professor of Pathology, for his advice and encouragement through this course of study.

Appreciation is extended to Doctor H. D. Anthony, Associate Professor of Pathology, and Doctor A. P. Gray, for providing some calves used in these studies.

Gratitude is extended to the personnel of the Department of Pathology, Parasitology and Public Health and specifically to the staff of the Veterinary Research Laboratory.

The author is indebted to the Office of the Surgeon General, Department of the Army, for selecting him for this course of study at Kansas State University.

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IMMUNOLOGIC STUDIES OF NATURAL AND EXPERIMENTAL  
CUTANEOUS STREPTOTHRICOSIS INFECTIONS IN CATTLE

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology, Parasitology  
and Public Health

KANSAS STATE UNIVERSITY  
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1965

The majority of the literature on streptothricosis concerns itself with the classification and description of the disease, its development and transmission.

In 1915, Van Saceghem first identified Dermatophilus congolensis as the causative agent of cutaneous streptothricosis.

The disease is clinically identified by an exudative dermatitis with extensive scab formation. The scabs are formed from coagulated exudate, epidermal cells, and filaments of the causative organism. Microscopically, the organism is seen in culture and lesions as cocci and filaments. The filaments are composed of rows of 0.5 to 1.0 micron coccoid elements in a gelatinous matrix. Numerous free motile cocci are seen in cultures and lesions.

This study was undertaken to evaluate the immunologic responses to Dermatophilus congolensis. In this study, agar gel precipitation, passive hemagglutination and agglutination tests were adapted to study the immunologic responses in cattle from natural and experimental infections of D. congolensis.

Six rabbits and 3 calves were hyperimmunized by repeated injections of lysed and whole cell suspensions of D. congolensis. Passive hemagglutination titers of 1:80 to 1:2560, agglutination titers of 1:160 to 1:640, and positive gel precipitation reactions were seen in the hyperimmunized animals. Increased titers were not observed in control animals.

Naturally infected and experimentally infected cattle had passive hemagglutination titers of 1:80 to 1:2560, agglutination

titers of 1:80 to 1:640 and positive gel precipitation reactions. Normal cattle had passive hemagglutination and agglutination titers of 1:10 to 1:40 and negative gel precipitation reactions.

Experimental streptothricosis infections were produced in cattle on areas which were chemically irritated and scarified before inoculation.